

Electronic Supplementary Material – Online Resource 1

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Article title: Caffeine inhibits PI3K and mTORC2 in *Dictyostelium* and differentially affects multiple other cAMP chemoattractant signaling effectors.

Authors: A.F.M. Tariqul Islam¹, Margarethakay Scavello¹, Pouya Lotfi¹, Dustin Daniel^{1,2}, Pearce Haldeman^{1,3}, and Pascale G. Charest^{1*}.

Affiliations:

¹ Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ, United States of America.

² Current address: Department of Basic Medical Sciences, University of Arizona, Phoenix, AZ, United States of America.

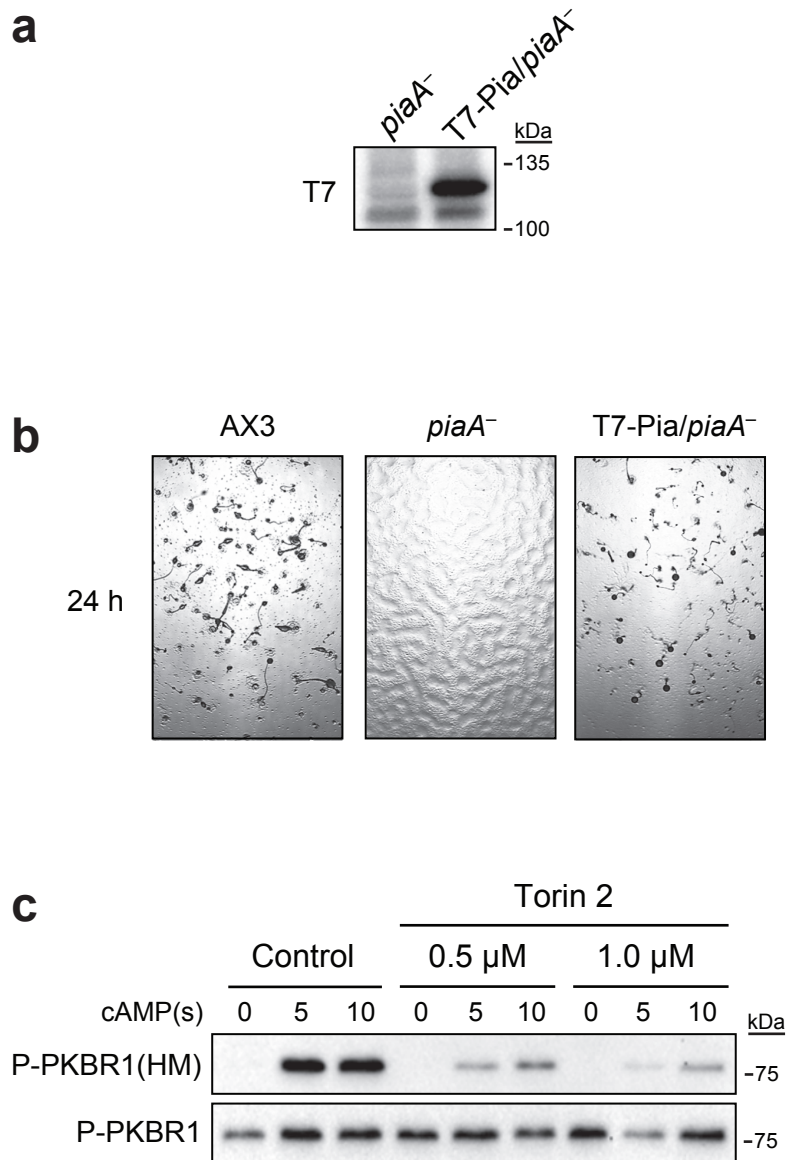
³ Current address: Division of Biology and Biological Engineering, Joint Center for Transitional Medicine, California Institute of Technology, Pasadena, CA, United States of America.

*** Corresponding author:** Pascale G. Charest

E-mail: pcharest@email.arizona.edu

Phone: 520-626-2916

ORCID (PGC): 0000-0002-2395-5294



Online Resource 1. a, T7-Pia was expressed in *piaA* null (*piaA*⁻) cells and detected by T7 immunoblot. **b**, T7-Pia rescues the developmental defect of *piaA* null cells, demonstrating that the construct is functional. Pictures shown were taken at 24 h after the onset of development triggered by starving the cells on non-nutrient agar. Fruiting bodies are seen on plates of wild-type AX3 and T7-Pia/*piaA*⁻ cells and absent on plates of *piaA*⁻ cells. **c**, Torin 2-mediated inhibition of mTORC2 in cells. Torin 2 was incubated with developed AX3 cells at 0.5 and 1.0 μM for 30 min before cAMP stimulation for the time indicated. mTORC2 activity was assessed by evaluating its phosphorylation of PKBR1 at its hydrophobic motif (HM).